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## ORIGINAL ARTICLE

# Influence of Hydro-priming duration on Field Performance of milk Thistle under Water Stress

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**ABSTRACT**
*A field experiment was conducted in 2015, to evaluate the effects of hydro-priming duration ($P_1$, $P_2$ and $P_3$: 0, 8 and 16 hours, respectively) on field performance of milk thistle (Silybum marianum L.) under different irrigation treatments ($I_1$, $I_2$, $I_3$, $I_4$: Irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively). Results showed that plant establishment per unit area increased with increasing priming duration up to 16 hours. Ground cover of plants from primed seeds was also enhanced under all irrigation treatments as a result of hydro-priming. Number of grains per plant, 1000 grains weight and biological and grain yields per unit area under all irrigation intervals improved with increasing seed priming duration. Increasing water limitation led to considerable reduction of these traits in $P_1$ and $P_2$ plants, but no significant decline in $P_3$ plants.These results clearly indicate that hydro-priming for 16 hours can largely reduce the deleterious effects of water stress on yield and yield components of milk thistle. The highest positive correlation with grain yield was recorded for biological yield, followed by ground cover, suggesting that these traits have the major roles in determining final yield of this medicinal plant.*
**Keywords:** *Grain yield, Ground cover, Hydro-priming,Milk thistle, Water stress*

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## INTRODUCTION

Plant products, as parts of foods or botanical potions have been used with varying success to cure and prevent diseases throughout some years [1].Milk thistle (*Silybum marianum* L.) is an annual or biannual plant of the Asteraceae family, which is commercially known as a medicinal plant in Europe, Egypt, China, Argentina and Iran.It has been also reported as a deleterious weed in some countries [2].Water availability may limit growth and development of this plant, depending on stress level.

In a large part of the agricultural areas in the world, water deficit is limiting crop productivity [3].Different techniques could be used to enhance crop yields, particularly under unfavorable stress conditions. Seed priming is a useful technique which can be used to improve seedling establishment and field performance of crops [4, 5]. Some of the deleterious effects of environmental stresses such as water limitation on crop performance may be reduced by seed priming [6]. Priming allows seed hydration to initiate the early events of germination, but not permit radicle emergence, followed by drying to initial moisture [4, 7]. Thus, primed seeds can rapidly imbibe and revive the seed metabolism (breaking of dormancy, enzyme activation and hydrolysis) resulting in rapid and uniform seed germination and seedling establishment. These are reportedly increase drought tolerance and crop yield [8]. The common methods of seed priming include osmo-priming, salt-priming, hydro-priming, matri-priming and thermo-priming [4, 9].

Hydro-priming is a simple method which markedly improved plant establishment and early vigor of mung-bean, resulting in faster development and higher yields [10].Priming by water enhances DNA replication and protein and RNA syntheses, alpha amylase activities and better embryo growth[4]. These events can improve germination rate, growth consistency, seedling vigor and establishment and consequently field performance of crops [11].Improvement in crop performance by hydro-priming was also reported for mung bean [10], lentil [12], chickpea [6] pinto bean[13], kenaf [14] and sunflower[15].The estimated optimal times of hydro-priming were 7 hours for pinto bean [16], 12 hours for chickpea [17] and 18 hours for maize [18]. However, the field responses of milk thistle to different hydro-priming durations, particularly under stressful conditions, were not documented. Thus, this research was undertaken to investigate the effects of hydro-priming duration on ground cover, seed yield and yield component of milk thistle under different irrigation treatments.

## MATERIALS AND METHODS

Seeds of milk thistle (*Silybum marianum* L) were obtained from Pakan Bazr, Esfahan, Iran. The experiment was conducted in 2015 at the Research Farm of the Faculty of Agriculture, University of Tabriz, Iran (Latitude 38 05′ N, Longitude 46 17′ E, Altitude 1360 m above sea level). The climate is characterized by mean annual precipitation of 245.75mm per year, mean annual temperature of 10°C, annual maximum temperature of 16.6 °C and mean annual minimum temperature of 4.2°C. The experiment was arranged as split-plot, based on randomized complete block design in three replications, with the irrigation treatments ($I_1$, $I_2$, $I_3$ and $I_4$: irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively) in main plots and hydro-priming durations of 0 ($P_1$), 8 ($P_2$) and 16 ($P_3$) hours were in sub-plots.

Seeds were divided into three sub-samples, one of which was kept as control (non-primed, $P_1$) and two other samples were soaked in distilled water at 20°C for 8 ($P_2$) and 16 ($P_3$) hours and then seeds were dried at 20-25 °C for 24 hours. These seeds were treated with 3.3g/kg Benomyl and then were sown by hand in 3 cm depth of a sandy loam soil on 7 May 2015. Each plot consisted of 6 rows of 3m length; spaced 25cm apart. All plots were irrigated immediately after sowing. After seedling establishment, irrigations were carried out according to the treatments. Weeds were frequently controlled by hand during crop growth and development.

Ground cover was measured by a wooden frame (50 × 50 cm dimensions) divided into 100 equal sections. The sections were counted when more than half filled with crop green area. At maturity, plants in 1 $m^2$ of the middle part of each plot were harvested and grain yield and yield components such as capitols per plant, grains per capitol, grains per plant and 1000 grain weight were determined. Then above ground biomass was oven-dried at 80˚C for 48 hours and biological yield per unit area was recorded. Analysis of variance of the data appropriate to the experimental design and comparison of means at $p \leq 0.05$ were carried out, using MSTATC and GenStat 12 software's. Excel software was used to draw figures.

## RESULTS AND DISCUSSION

Plant establishment significantly affected by hydro-priming duration ($p \leq 0.01$).The number of plants per unit area increased with increasing priming duration up to 16 hours (Figure 1).This result suggests that hydro-priming for 16 hours is the best duration for seed invigoration and seedling emergence of milk thistle. The efficiency of seed hydro-priming for better seedling emergence is also reported in barley [19], lentil [5] and chickpea [6].

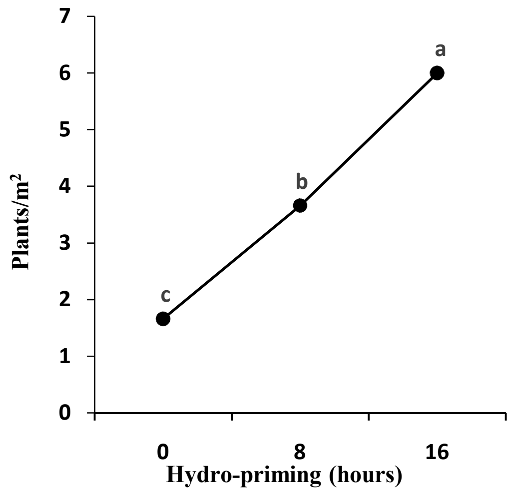


**Figure 1.** Mean plants/$m^2$ of milk thistle affected by hydro-priming duration
Different letters indicating significant difference at $p \leq 0.01$.

Percentage ground cover significantly affected by both water limitation and hydro-priming duration. Interaction of water limitation × hydro-priming duration was also significant for this trait (Table 1). Ground cover of plants from primed seeds was also enhanced with increasing hydro-priming duration under all irrigation treatments. Ground cover of plants from unprimed seeds decreased with decreasing water supply, but hydro-priming prevented significant reduction in ground cover due to water limitation(Table 2). These results directly related with increment in plant establishment as a result of prolonging priming duration (Figure 1).Optimum ground cover is essential for the efficient use of resources like water and light [20-21, 6].Hydro-priming proved to be a useful way to overcome some deleterious effects of water stress on ground cover of milk thistle. Similar results have been reported for barley [22], borage [12] and mung bean [10]. Thus, ground cover is a reliable index to estimate yield potential of the plants under favorable and adverse environmental conditions [23].

Interaction of water supply × hydro-priming duration was also significant for biological yield (Table 1).Biological yield under all irrigation intervals improved as a result of enhancing seed priming duration. Increasing water limitation led to considerable reduction in biological yieldof $P_1$ and $P_2$plants, but no significant decline in biomass of $P_3$ plants, indicating that hydro-priming for 16 hours is a simple method that can largely inhibit reductions in biological yield under water stress. These variations in plant biomass were strongly associated with changes in ground cover under different treatments (Table 2).Reductions of percentage and duration of ground green cover due to water stress, can reduce the absorption of incident PAR, either by drought-induced limitation of leaf area expansion, or by early leaf senescence [24], which can potentially reduce photosynthesis and consequently biological yield [10].

**Table 1.** Analysis of variance of field traits of milk thistle affected by water supply and hydro-priming duration.

| Source of Variation | Df | MS | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ground cover | Biological yield | Grains per plant | 1000 grain weight | Grain yield | Harvest Index |
| Replication | 2 | 7.00 | 562645 | 4650 | 0.01361 | 142.0 | 0.0753 |
| Irrigation (I) | 3 | 125.78* | 18911647** | 2850968** | 0.73657** | 7680.8** | 5.5370** |
| Error | 6 | 13.67 | 282228 | 9582 | 0.00880 | 90.9 | 0.0601 |
| Hydro-priming (P) | 2 | 15960.58** | 96545826** | 100163* | 0.04111* | 47586.3** | 24.3536** |
| I × P | 6 | 52.25* | 2969881** | 823398** | 0.27519** | 1219.2** | 2.0206** |
| Error | 16 | 18.75 | 466470 | 25427 | 0.01083 | 194.9 | 0.1672 |
| CV (%) | - | 6.7 | 14.5 | 12.6 | 0.5 | 14.3 | 2.0 |

ns, * and **: No significant and significant at p≤0.05 and p≤0.01, respectively

**Table 2.** Means of ground cover, yield components and grain yield of milk thistle affected by water supply and hydro-priming duration

| Treatments | | Ground cover (%) | Biological yield ($g/m^2$) | Grains per plant | 1000 grains weight (g) | Grain yield ($g/m^2$) | Harvest Index (%) |
|---|---|---|---|---|---|---|---|
| $I_1$ | $P_1$ | 35.00c | 469.7c | 2447.0a | 22.80a | 93.00c | 19.73de |
| | $P_2$ | 73.33b | 686.8b | 1680.0b | 22.37b | 137.20b | 20.07cd |
| | $P_3$ | 98.33a | 821.4a | 1316.0c | 22.17bcd | 175.30a | 21.33ab |
| $I_2$ | $P_1$ | 28.67cd | 244.4d | 2177.0b | 22.30bc | 48.50d | 19.87d |
| | $P_2$ | 75.67b | 521.0c | 1611.0b | 22.27bc | 107.60c | 20.67bc |
| | $P_3$ | 97.67a | 770.2ab | 1235.0c | 22.23bc | 164.80a | 21.40ab |
| $I_3$ | $P_1$ | 21.67cd | 58.0e | 481.0e | 21.80e | 10.50e | 18.13f |
| | $P_2$ | 71.00b | 277.7d | 877.0d | 22.03d | 58.00d | 20.90b |
| | $P_3$ | 96.00a | 717.5ab | 1176.0c | 22.23bc | 156.90ab | 21.87a |
| $I_4$ | $P_1$ | 15.67d | 60.6e | 357.0e | 21.40f | 10.00e | 16.57g |
| | $P_2$ | 71.33b | 252.0d | 605.0de | 21.83e | 48.10d | 19.07e |
| | $P_3$ | 95.67a | 778.3ab | 1178.0c | 22.13cd | 164.00a | 21.07b |

Different letters in each column indicate significant difference at p ≤ 0.05.

$I_1$, $I_2$, $I_3$, $I_4$: Irrigations after 70, 100, 130 and 160 mm evaporation, respectively.

$P_1$, $P_2$, $P_3$: Hydro-priming for 0, 8 and 16 hours, respectively

Grains per plant and 1000 grains weight were significantly influenced by interaction of water limitation × hydro-priming duration (Table 1).The number of grains and 1000 grains weight of $P_1$ and $P_2$ plants decreased with decreasing water availability. However, water stress had no significant effect on grains number and weight of $P_3$ plants. Grains per plant Under $I_1$ and $I_2$ decreased, but under $I_3$ and $I_4$ increased as a result of seed hydro-priming(Table 2). These results clearly show the advantage of hydro-priming particularly for 16 hours under stressful conditions. Because of higher population density of plants from primed seeds (Figure 1), this can potentially improve grain yield per unit area of these plants under different irrigation intervals.

The interaction of irrigation × hydro-priming duration was also significant for grain yield per unit area and harvest index (Table 1). Grain yield per unit area of $P_1$ and $P_2$ plants decreased with decreasing water supply, but grain yield of $P_3$ plants did not change significantly under limited irrigation conditions. Harvest index of plants from unprimed seeds considerably reduced under $I_3$ and $I_4$, but that of $P_2$and $P_3$ plants only slightly declined under severe water stress. The superiorities of plants from hydro-primed seeds in grain yield per unit area and harvest index were much greater under moderate ($I_3$) and severe ($I_4$) water limitation (Table 2).The superiority of plants from hydro-primed seeds, especially for 16 hours, in grain yield per unit area could be attributed to high stand establishment (Figure 1), ground cover and biological yield under different moisture availability (Table 2). Similar advantages in grain yield of plants from hydro-primed seeds, but for lesser extent, were also reported for upland rice [25], chickpea [6]and lentil [12]. The optimal times of hydro-priming for pinto bean [16], chickpea [17] and maize [18] were 7, 16 and 18 hours, respectively. The best duration for hydro-priming of milk thistle seeds was 16 hours (Table 2).

Correlations of ground cover, biological yield and harvest index with each other and with grain yield were positive and significant. However, grains per plant and 1000 grains weight were positively, but not significantly, correlated with grain yield. The highest positive correlation with grain yield was recorded for biological yield, followed by ground cover (Table 3). This suggests that ground cover and biological yield have the major roles in determining final grain yield of milk thistle, similar to that reported for rapeseed [26] and borage [8].

**Table 3**. Correlation coefficients of different field traits

| | Ground cover | Biological yield | Grains per plant | 1000 Grain weight | Harvest Index | Grain yield |
|---|---|---|---|---|---|---|
| Ground cover | 1 | | | | | |
| Biological yield | 0.86** | 1 | | | | |
| Grains per plant | 0.03 | 0.38 | 1 | | | |
| 1000 Grain weight | 0.27 | 0.56 | 0.91** | 1 | | |
| Harvest Index | 0.85** | 0.82** | 0.41 | 0.62* | 1 | |
| Grain yield | 0.87** | 0.99** | 0.36 | 0.54 | 0.84** | 1 |

*, **: Statistically Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

## CONCLUSION

Seed hydro-priming is a simple and environmentally friendly technique for improving field performance of milk thistle, particularly under water stress. The optimal duration for seed hydro-priming of this medicinal plant is 16 hours. Plant biomass and ground green cover highly and positively correlated with grain yield per unit area, suggesting that improving these traits can considerably enhance final yield of milk thistle.

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